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Lipolytic Activity of Some Pathogenic Microbes

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(Submitted to Editorial Board 18/VII 1961)

The problem concerning the esterases of pathogenic microbes has been weakly covered in literature up to now. This can be conjectured on, if only about the lack of appropriate facts in one of the recent reviews about lipase in animals, plants and microorganisms (Novitskaya, 1960). Only lecithinase (phosphatidase) constitutes an exception, which is explained by its role in the pathogenesis of several diseases, for example gas gangrene. Besides this, the presence of detailed information about the esterases of pathogenic microbes as well as about other fermentation systems can considerably increase the potentialities during the formulation of a bacteriological diagnosis, and promote the development of microchemical methods of identifying bacteria.

The goal of the present investigation resulted in the exposure of one of the esterases lipase - in a number of pathogenic microorganisms and the study of its properties.

We determined lipolytic activity by the method of Michaelis and Nakahara (1923). This method was selected as one of the most sensitive and expedient in working with pathogenic microbes. Another method - the titrimetric method - gave worse results under our conditions.

We prepared the substrate solution by means of a half hourly vibration in a vibrating device of 10 drops of tributyrin with 100 ml of an M/15 phosphate buffer with a pH of 7.0. Then we filtered the emulsion of tributyrin through paper moistened with water or buffer

Plague (avirulent strains EV and N 17), pseudotuberculosis (virulent strains No. 1 and 12), and anthrax (STI strain) bacilli were incubated on Hottinger agar with a pH of 7.2. Brucellae (Br. suis strain No. 1330, Br. melitensis strain No. 16, Br. abortus strain No. 544 and BA Br. rangiferis,[1] strains No. 8, 20, 184, 20/8993) were incubated on a medium prepared from dry agar with a pH of 6.8, and the tularemia microbe (virulent strains No. 220, 221, 279, 280, 347 and avirulent strain No 15) - on Antsiferov's medium (1954). The bacteria were incubated at a temperature optimal for each species for a period of 48 hours.[2] The cells were washed from the medium and rinsed three times

with a phosphate buffer by means of centrifugation at 3×10^3 revolutions per minute. From the bacteria that was washed off, a suspension was prepared containing 5×10^{10} microbial cells per ml. Then 2 ml of the bacterial suspension was mixed with 8 ml of a tributyrin solution and immediately the number of drops was determined. Then the specimens, as a rule, were placed in a thermostat at 37° and the number of drops repeatedly computed after specified time intervals. Specimens with microbes killed by heating served as a control.

By this method we determined the lipolytic activity in lyophilized microbes even in a hypersedimentary liquid after sedimentation of the bacteria by centrifugation. In all cases we judged the amount of split tributyrin by a calibration curve.

Lipase was detected in the anthrax microbe, virulent and avirulent strains of the causative agent of tularemia, and all types of brucella. The enzyme was lacking in plague and pseudotuberculosis bacilli. The last fact supports the unpublished results of our previous observations of a great number of strains and is in complete conformity with the weakly expressed hydrolytic capability of these two microorganisms in relation to other substrates (Domaradskiy, 1958).

The greatest lipolytic activity was found in the tularemia microbe which completely split tributyrin even at room temperature in 10-12 minutes. The speed at which the two other species of bacteria split tributyrin was considerably lower: In order for them to completely split the substrate, incubation was required for several hours at 37°. The drawing (page 15) supports what has been said. On it a graph has been constructed to a semi-logarithmic scale based on the average data of several computations. The tangent of the angle of inclination of the lines to the axis of abscissa characterizes the constant of the reaction rate (K/2303).

Judging by the data presented, the splitting of tributyrin by microbes is a reaction of the first order.

On the basis of the constants for the reaction rate, we calculated the time for the half-decomposition of the substrate in hours (t1/2). For all the strains of the tularemia microbe the t1/2 didn't exceed 0.025, for the anthrax bacilli it was equal to 0.78, and for the various types of brucella it fluctuated from 2.3 (<u>Br. abortus BA</u>) up to 5.6 (<u>Br. melitensis</u>, strain No. 16).

In vaccine strains of tularemia and anthrax causative agents, the lipolytic activity was preserved even after lyophilization of the microbes, however in dessicated cells it was somewhat lower (the t1/2 of the first one equaled 0.041, and the second - 1.62).[3]

From the point of view of comparative microbiology, the fact is

interesting that, based on lipolytic activity, the avirulent strain of anthrax bacillus used by us didn't differ from Bacillus anthracoides. In 2 strains of the latter non pathogenic microbe, the average value of t1/2 equaled correspondingly 0.69 and 0.7, and in lyophilized cells of these strains - 1 73 and 1.75.

Only in one microbe - the tularemia causative agent - was lipase apparently discharged from the cell: The hypersedimentary liquid, after sedimentation of the microbes by centrifugation for an hour at 12 x 10^4 revolutions per minute, still split tributyrin with a speed exceeding that at which tributyrin was split by washed off cells of other species of bacteria (t1/2 = 0.37). However, it must be remembered that the tularemia microbe belongs to the ranks of the most minute microorganisms and is precipitated by centrifugation with great difficulty. Therefore, with the aim of the strictest proof of the capability of the tularemia microbe to discharge lipase into the surrounding medium, further investigations are necessary. The fact itself of the extremely high lipolytic activity of the tularemia microbe merits particular attention since microbiologists treat it as one of the "biochemically inactives". It is possible that the presence of lipase is the result of cultivation of the given species of microorganism on media with egg yolk which are elective media for it

Subsequent experiments showed that optimum lipase activity of washed cells of anthrax and tularemia microbes occurred within the limits of pH 6.5 to 7.0. In contrast to this, the optimum of lipase activity of several other species of microorganisms was found at higher pH values (Michaelis and Nakahara, 1923; Gorbach with co-authors, 1955). Only the lipase of Pseudomonas fragi is optimum for the splitting of coconut oil at a pH of 7.0 (Nassif and Nelson, 1953).

In the pH limits specified by us, enzyme activity under equal conditions was almost twice as high in phosphate and citric-phosphate buffers than in acetate-veronal and borate buffers. Citrate stimulation of the lipase of bacterial derivation was also noted by Michaelis and Nakahara (1923).

When the concentration of tularemia microbes was decreased 8 times, the period of half-decomposition of the tributyrin increased 16 times; with the same decrease in concentration of anthrax microbes, the period of half decomposition of the substrate increased 12 times.

We formed an opinion about the influence of temperature on the speed of the reaction according to the temperature coefficient and energy of activation. We conducted the determination of the temperature coefficient at temperatures of 4, 14, 24, and 37°. From the results obtained it follows that with an increase of temperature, the speed of tributyrin splitting is increased. For example, the speed of the reaction of tributyrin splitting by washed cells of anthrax microbes

at 24-37° was approximately 3 times higher than at 4-24°. The energy of activation, computed for the maximum speed of the reaction, turned out to be equal to 9600 in case of tributyrin splitting by a tularemia microbe, and 22,500 cal/mol in the event of splitting by the anthrax causative agent.

Comparative data concerning the lipolytic activity of various types of brucella will be presented later.

Conclusions

1. Lipase was detected in a vaccine strain of the anthrax microbe, virulent and avirulent strains of the tularemia causative agent and all types of brucella. The greatest lipase activity was found in the tularemia microbe which apparently easily discharges this enzyme into the surrounding medium. Lipase was lacking in vaccine strains of the plague microbe and virulent strains of the causative agent of pseudotuberculosis of rodents.

2. The pH optimum for lipase activity of the causative agents of tularemia and anthrax was between 6.5 - 7.0. The speed of tributyrin splitting in phosphate and citric-phosphate buffers was twice as fast as in acetate-veronal and borate buffers. With an increase of temperature from 4° up to the optimum, the speed of the reaction increased almost 3 times.

3. Lipase activity was preserved even after lyophilization of the cells.

Footnotes

1. (page 14) According to A. F. Pinigin (1960) - this is a new type of brucella isolated from northern deer.

2. (page 14) By the end of the 2 day period, under the influence of bacteria which form lipase, there is usually observed the maximum rate of tributyrin splitting (Lembke with coauthors, 1954).

3. (page 15) We didn't lyophilize virulent strains of the tularemia microbe and brucella.

Diagram (page 15)

A - by the tularemia microbe
B - by the anthrax bacillus
V - by brucella
C - course of the reaction in time: splitting of tributyrin.
D - length of incubation (in hours)
E - splitting of tributyrin (8%)
F - lg

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2. Domaradskiy, I. V., (Ibid) ... Illegible...... t. 18, p 155

3 Novitskaya, G. V., Advances of Modern Biology, 1960, t. 50, v. 1, p. 29.

4. Pinigin, A. F., Petukhova, O. S., News of the Irkutsk Scientific Research Antiplague Institute for Siberia and the Far East, 1960, T. 23, p. 346.

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Summary

(This is the English summary which appears with the Russian original.)

Lipase was revealed in the vaccine strain of the anthrax bacillus, virulent and avirulent strains of F. tularensis and of all the brucella types. The greatest lipase activity was possessed by F. tularensis, which apparently easily discharged this enzyme into the surrounding environment. Lipase was absent in the vaccine strains of Past. pestis and in the virulent strains of Bacillus pseudotuberculosis of rodents The optimal action of lipase of the F. tularensis and anthrax bacilli lies within the pH range of 6.5 - 7.0. The rate of tributirin splitting in the phosphate and the citric-phosphate buffers was twice as more as in the acetate-veronal and borate buffers. With the rise of temperature from 4°C to the optimal the reaction was accelerated almost three-fold.

The lipolytic activity was retained in the microbes after their lyophilization.